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Epigenetic Patterns of PTSD: DNA Methylation in Serum of OIF/OEF Service Members

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The molecular mechanisms involved in PTSD are not well characterized; epigenetic factors could offer new insights. Gene expression studies have found differences in cytokine expression signatures between PTSD patients and controls. DNA methylation is intrinsically linked to the regulation of gene expression and is modifiable. We are carrying out a systematic investigation of DNA methylation patterns in cytokines in soldiers prior to OIF or OEF deployment; serum derived DNA is being used. PTSD cases with existing serum samples housed at the Department of Defense Serum Repository (DoDSR) were identified using the criteria of having experienced an OIF/OEF deployment and having an outpatient record with a primary diagnosis of PTSD, based on ICD-9 codes; an appropriate control group was identified. For each PTSD case and each control, AFHSC pulled a pre- and post-deployment serum sample. DNA will be extracted from each serum sample and methylation quantified via pyrosequencing for promoter regions of the following cytokines: IL-8α, IGF2, IL-18, IL-16, and EDG1 and in long interspersed nucleotide elements, which represent global genomic methylation. We will make statistical comparisons via t-tests and linear regressions for patterns of DNA methylation between cases and controls and between pre- and post-deployments of each group. We will also measure levels of these specific cytokines using an ELISA approach. At this time we are in the process of extracting the DNA from serum samples of the PTSD cases and controls.

**15. SUBJECT TERMS**
PTSD, epigenetics, DNA methylation, cytokines, serum, pre-deployment, post-deployment

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## Introduction

As the conflict in Iraq continues, public health authorities in the United States anticipate that many returning soldiers will suffer from post-traumatic stress disorder (PTSD). A recent study of combat troops following return from deployment found PTSD rates to range from 12.2%-12.9%. [1] The underlying molecular mechanisms or outcome predictors determining these differences are not known. Epigenetic factors - inherited and acquired modifications of DNA and histones that regulate various genomic functions occurring without a change in nuclear DNA sequence - could offer new insights about PTSD. Profiling has been done using peripheral blood during the triggering and development of PTSD to measure gene expression of trauma survivors at the emergency room and 4 months later. [2, 3] Using cDNA microarrays, investigators have found differential gene expression signatures in cytokines which distinguished PTSD patients from those who met no PTSD criterion. [2, 3] Aberrant DNA methylation patterns have also been found in other psychiatric disorders. [4, 5] However, to date our understanding of the epigenetic mechanisms influencing gene expression underlying PTSD is minimal. An epigenetic mechanism, DNA methylation may play a significant role in the pathophysiology of PTSD, since the process is intrinsically linked to the regulation of gene expression.

We proposed a systematic investigation of DNA methylation patterns in the promoter regions of a group of cytokines, IL-8α, IGF2, IL-18, IL-16, and EDG1 and in genomic repetitive elements (representing global genomic methylation), in soldiers prior to Operation Iraqi Freedom (OIF) or Operation Enduring Freedom (OEF) deployment/PTSD diagnosis, and post deployment/PTSD diagnosis, and an appropriate group of controls. Since human studies of brain tissue are highly invasive and in many cases impractical, we argued that it would be of great value to identify a low-invasive biomarker of epigenetic patterns of PTSD. We are currently carrying out this study in DNA extracted from serum samples housed at the Department of Defense Serum Repository (DoDSR). We are also investigating cytokine levels of a panel of 24 cytokines in a subset of these sera.

Our study is innovative in that we are investigating epigenetic mechanisms of PTSD in human serum samples, an effort not previously carried out, we are investigating serum as a biomarker of epigenetic patterns of PTSD, we will utilize a quantitative method to measure DNA methylation, and we are using serum from a large serum bank which houses longitudinally collected samples.

*Objectives:*

- **(1)** Compare DNA methylation patterns, both gene-specific promoter region and global, between PTSD cases and an appropriate group of controls, post-OIF/OEF deployment
- **(2)** Compare DNA methylation patterns, both gene-specific promoter region and global, between PTSD cases and an appropriate group of controls, pre-OIF/OEF deployment
- **(3)** Compare DNA methylation patterns, both gene-specific promoter region and global, for PTSD cases between two periods of serum collection: 1. pre-OIF/OEF deployment and 2. post-OIF/OEF deployment

*Significance:*

This study will help elucidate the molecular sequelae of PTSD. It will be the first study to investigate an epigenetic mechanism, specifically DNA methylation associated with PTSD. The

study of the role of DNA methylation in the disease process of PTSD has the potential to transform our understanding about the molecular etiology of this complex disease. Understanding the differential roles of regional hypermethylation and global hypomethylation in PTSD will fuel novel therapeutic approaches to PTSD therapy, particularly since modifications in DNA methylation can potentially be reversed.

Additionally, there is a need to develop sensitive and specific biomarkers designed to detect epigenetic mechanisms involved in PTSD, however, to date such biomarkers are still not well defined. The results of this small molecular epidemiology study will lay the foundation for future epigenetic studies based on the longitudinally collected serum samples (multiple samples per service member) housed at the DoDSR, a vast resource of bio-specimens which can be linked to detailed demographic, deployment, and medical data.

**Body**

*Methods*
The Department of Defense Serum Repository (DoDSR) stores serum that remains following mandatory HIV testing and specimens related to operational deployments worldwide among the active and reserve components of the U.S. military. The DoDSR currently houses nearly 40 million specimens and grows by approximately 2.3 million specimens per year. The availability of serial serologic specimens, as well as relevant demographic, deployment, and medical information within the databases at the Armed Forces Health Surveillance Center (AFHSC) enables the DoDSR to make significant contributions to molecular epidemiologic investigations.

PTSD cases (n=75) with existing serum samples housed at the DoDSR were identified by AFHSC using the criteria of having experienced an OIF/OEF deployment and having an outpatient record with a primary diagnosis of PTSD, based on coding from the International Classification of Diseases, 9th Revision (ICD-9 Code = 309.81). The study population is defined as below:

<u>Cases and Controls</u>

- Among active duty Army and Marines with at least 2 years of continuous AD prior to OIF/OEF deployment
- Ever had at least 1 deployment to OIF/OEF for 6-18 months
- Pre-1st OIF/OEF deployment, absence of any mental health diagnosis
    - No psychological conditions as per ICD9 codes 290-320 dating back to at least 2 years prior to OIF/OEF deployment
- Post-1st OIF/OEF deployment, absence of any of the following mental health diagnoses: schizophrenia(ICD9 code 295), bi-polar disorders (ICD9 code 296), manic phase bipolar disorder (also ICD9 code 296): diagnoses EVER (either inpatient or outpatient)

<u>Cases</u> (n=75; 25 women, 50 men) (total samples = 150; 1 pre-, 1 post-deployment)

- Focus on 1st deployment
- With 2 outpatient diagnoses of chronic PTSD (ICD9 Code=309.81) in the 1st diagnostic position.

- 1st outpatient diagnosis within 4 to 12 months post deployment RETURN
- 2nd outpatient diagnosis, after that but within 2 years post deployment RETURN
- With 1 serum sample drawn within 12 months prior to deployment AND
- With 1 serum sample drawn within 6 months post deployment RETURN
- Random sample of age on first day of 1st deployment 20-35 and race (white, black)

Controls (n=75; 25 women, 50 men) (total samples = 150; 1 pre-, 1 post-deployment)

- Focus on 1st deployment
- With NEVER having had diagnosis of PTSD (ICD9 Code=309.81) or TBI ICD9 Codes=800.0-801.9, 803.0-804.9, or 850.0-854.1)
- With 1 serum sample drawn within 12 months prior to deployment AND
- With 1 serum sample drawn within 6 months post deployment RETURN
- Frequency match controls to cases on gender, race (white, black), and age group (20-26, 27-35)

We identified an appropriate control group (n=75), who were randomly selected from among those service members who met the same deployment, age, and serum sample criteria as cases, but for whom there was never a diagnosis of PTSD.

Data requested for each subject:

- PTSD case/control status
- age (in years) on 1st day of 1st deployment
- gender
- race (White, Black)
- date of 1st deployment start (month/year)
- date of 1st deployment end (month/year)
- date of 1st blood draw (month/year)
- date of 2nd blood draw (month/year)
- Any TBI diagnoses (ICD-9 codes 850.0 – 854.1) until 6 months post 1st deployment

For each PTSD case and each control, AFHSC identified a pre-deployment serum sample and a post-deployment serum sample (total $n_{samples} = 300$). DNA is currently being extracted from each serum sample and DNA methylation will be quantified via bisulfite treatment, PCR, and pyrosequencing. Specifically, percent methylated cytosine (%mC) will be quantified in the promoter regions of the following cytokines, IL-8α, IGF2, IL-18, IL-16, and EDG1 and in long interspersed nucleotide elements, which represent global genomic methylation. We will also measure cytokine levels in sera in a subset of both cases and controls for a panel of 24 cytokines. We will make statistical comparisons via t-tests and linear regressions for patterns of DNA methylation and for cytokine levels between cases and controls and between pre- and post-deployments of each group.

**Key Research accomplishments**

To date, we have identified PTSD cases and controls and have received the serum samples corresponding to each case and control (both pre- and post-OIF/OEF deployment) from the

DoDSR. We are just starting to extract DNA and to run cytokine assays on a subset of the sera in our lab.

**Reportable outcomes**

None, to date.

**Conclusions**

N/A

**References**

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